Exploring the Antitumorigenic Effects of Conjugated Linoleic Acids:
A literature review

Whitney Lundy
CWID 11447757
University of Alabama
NHM 454 Experimental and Functional Food Science
February 14, 2014
Introduction
Conjugated linoleic acids (CLA) are a group of geometric and positional isomers of linoleic acid, an omega-6 fatty acid. CLA can be biosynthesized from natural plant sources, such as mushrooms, and ruminant animal sources such as dairy and meat. CLA can also be synthesized in a laboratory setting for use in supplements and functional foods.

CLA have received much attention for supposed health benefits, including anti-atherogenic, anti-obesitic, apoptotic, and immunomodulatory effects. Also notable are the potential antitumorigenic properties of CLA. This review examines CLA’s potential impact on inflammatory markers associated with breast cancer and rectal cancer when administered as a supplement prior to and during treatment.

Science behind Conjugated Linoleic Acid

Linoleic acid is an 18-carbon essential fatty acid, possessing two double bonds at the 9 and 12 carbon atoms that are separated by two single bonds. CLA isomers result when the two double bonds are no longer separated by two single bonds. More than two dozen isomers exist, but the most abundant forms are the cis-9, trans-11 and trans-10, cis-12 configurations. These two isomers are the most frequently utilized in research.

CLA are synthesized naturally in the digestive tract and absorbed into the tissues of ruminant mammals – mammals like cows, goats and sheep which acquire nutrients from plants by bacterially fermenting the food in a specialized stomach called a rumen prior to digestion. Grass-based diets improve CLA concentration in total saturated fatty acid composition of animals; grain-based diets produce lower concentrations of beneficial CLA isomers.¹

CLA isomers can also be found in mushrooms, or synthesized from vegetables oils with high levels of linoleic acid such as safflower, sunflower, canola, soybean, and corn.² Dietary supplementation of animals can significantly raise the CLA concentration in their milk and meat.
fat. Meat fat CLA concentration can increase by 75% when the finishing diet of cattle is supplemented with sunflower and soybean oils.\(^3\)

The FDA granted GRAS (generally recognized as safe) status to CLA for use as a food additive in fruit juices, meal replacement bars and drinks, yogurt, liquid and powdered cream substitutes, and milk chocolate.\(^4\) CLA may be added to these designated food products in amounts up to 1.5g per serving. CLA are also available as stand-alone dietary supplements; popular brands include Tonalin\(^\text{®}\) and Clarinol\(^\text{®}\), and are sold in a variety of forms, including soft chews, capsules, and powder formulas. CLA are lipophilic, so they rely upon emulsifiers for incorporation into water-based products.

CLA isomers have been shown to induce a number of alterations to cellular and biochemical characteristics and functions, including cell proliferation and apoptosis; cell signaling; growth factors; prostaglandin, eicosanoid, and fatty acid metabolism; and inhibition of tumorigenesis in colon, mammary, prostate and skin cells.\(^5\) The mechanisms responsible for the effects of CLA remain rather unclear, and are currently the focus of many studies.

Concerns regarding the safety of CLA have been expressed in numerous studies, though results are conflicting. A 2009 review by Benjamin and Spener\(^6\) identified potential negative effects of CLA intake in human subjects; \textit{trans}-10, \textit{cis}-12 CLA may promote colon carcinogenesis, and may induce hyperproinsulinemia in obese individuals. Isomer specificity and ratios in CLA test samples may play a role in the conflicting research. The \textit{t}10, \textit{c}12 isomer is associated with more of the detrimental effects than the \textit{c}9, \textit{t}11 isomer; an equal ratio of the two isomers is believed to offset the detrimental effects of \textit{t}10, \textit{c}12 CLA.\(^6\)

\textbf{Highlighting Primary Research, Article I}

From June 2009 to March 2011, McGowan et al. conducted a single arm, open-label, single-institution proof of principle study to examine the effects of short-term CLA supplementation in
human breast tumors. Primary objectives were to determine if 10 or more days of administering CLA suppresses Spot 14 (S14), lipoprotein lipase (LPL), or fatty acid synthase (FASN) expression, and to define CLA’s effect on biomarkers of apoptosis and tumor cell proliferation.

Study participants were women with invasive non-meta-static breast cancer, selected based on strict inclusion and exclusion criteria determined to effectively test the study hypothesis. Clarinol® gel capsules containing 750mg of a 50:50 ratio of \( c_9, t_{11} \) and \( t_{10}, c_{12} \) isomers were taken twice daily by the participants for a minimum of 10, but up to 28 days. Fasting blood samples were obtained prior to treatment, and again the morning of surgery. Breast tissue samples from surgery were analyzed via immunohistochemistry to assess FASN, LPL, and S14 expression. The CLA capsules acted as the independent variable in this study, with immunohistochemical scores of FASN, LPL and S14 acting as the dependent variables.

Pre-supplementation, mean concentrations of free \( c_9, t_{11} \) and \( t_{10}, c_{12} \) CLA in plasma were measured at 0.11 ± 0.02 mg/L and 0.58 ± 0.07 mg/L, respectively. Post-supplementation, concentrations of the two isomers were 1.10 ± 0.16 mg/L and 2.17 ±0.08 mg/L, respectively. Tumor proliferation was estimated by calculating the percentage of cells expressing Ki-67. Baseline proliferation ranged from 2.6 to 33.0%. Post-treatment proliferation ranged from 3.7 to 23.5%, with 16 cases showing a decline in Ki-67 expression. Ki-67 is of importance, as high levels of this antigen can indicate an aggressive tumor with a poor prognosis for the patient.

Immunohistochemical (IHC) staining for the 3 proteins (S14, LPL, FASN) was graded 0, 1, or 2. Pre-treatment S14 IHC scores were as follows: 0 grade 0, 11 grade 1, and 13 grade 2. Post-treatment IHC scores for S14 were: 0 grade 0, 22 grade 1, and 2 grade 2. While none of the 11 grade 1 tumors declined with treatment, 11 of the grade 2 tumors declined with treatment. IHC scores for FASN and LPL showed no statistically significant change with treatment.
These results suggest that preoperative supplementation of 10 or more days with 7.5g daily of CLA can significantly reduce Ki-67 and S14 expression in primary invasive breast cancer tissue. IHC scores for S14 suggest that initial metabolic status of the cancer cells may dictate their reaction to CLA supplementation at the tested dose.

**Highlighting Primary Research, Article II**

In 2013, Mohammadzadeh et al. published research that examined the effect of CLA supplementation on inflammatory factors and matrix metalloproteinase in rectal cancer patients undergoing radiochemotherapy. The randomized, double-blind, placebo-controlled pilot study enrolled 34 volunteer patients diagnosed with rectal cancer who were selected based on strict inclusion and exclusion criteria determined to effectively test the study hypothesis.

Patients were blindly assigned to either the CLA group or the placebo group. The CLA group received 4 1000-mg capsules that provided a total of 3g CLA daily in a 50:50 ratio of c9, t11 and t10, c12 isomers. Placebo capsules containing sunflower oil were provided to the placebo group. Placebo and CLA capsules were to be taken 3 times daily – 1 capsule at breakfast, 2 capsules at lunch, and 1 capsule at dinner. Patients started supplementation 1 week prior to beginning radiotherapy, continuing the regimen every day until the conclusion of radiotherapy.

Patients were monitored weekly for potential CLA side effects. Bi-weekly capsule counts were conducted to monitor compliance. Fasting blood samples were obtained prior to and upon completion of the intervention. From the blood samples, 9 serum biochemical factors were assessed: TNF-α, IL-1β, IL-6, hsCRP, MMP-9, MMP-2, ALT, AST, and ALP. The CLA capsules acted as the independent variable in this study; the biochemical factors assessed were the dependent variables.

No significant differences in baseline characteristics between the control group and treatment group were observed. After treatment, significant changes were noted in the CLA group’s TNF-α
levels, compared to the control group. Mean serum hsCRP levels and ALP concentration decreased for the CLA group, but not for the control group. The CLA group also showed reductions in MMP-2 and MMP-9 that were significant when compared to the control group.

Inflammation is believed to impact the progression, metastasis, and resistance of colorectal cancer, which is why this study examined CLA’s effect on key inflammatory factors. Study results indicate that CLA supplementation improved inflammatory markers hsCRP and TNF-α, and reduced the enzymes MMP-2 and MMP-9, which act as biomarkers for tumor invasion and atherogenesis.

**Highlighting Primary Research, Article III**

Beginning in 1987, Larsson, Bergkvist and Wolk conducted a prospective cohort study of 61,433 Swedish women. The study objective was to examine the association between intake of CLA and the incidence of invasive breast cancer. Study participants were selected based on strict inclusion and exclusion criteria determined to effectively test the study hypothesis.

Cohort participants completed 67-item and 96-item food frequency questionnaires at the start and ten years later in 1997. Nutrient intakes were then calculated from the completed FFQs. CLA intake was estimated from published values of CLA concentrations in various foods. National and regional cancer registries provided data on invasive breast cancer incidences from cohort participants, which were histologically confirmed. Estrogen receptor (ER) and progesterone receptor (PR) status of confirmed breast tumors were obtained via clinical databases and laboratory reports. ER and PR status were examined via immunoassay and immunohistochemical methods.

In this study, the independent variable was CLA intake, and the dependent variable was breast cancer risk. The mean baseline CLA intake of study subjects was 118.4 ± 47.0 mg/d. During a mean 17.4 year follow-up of participants, 2,952 cases of invasive breast cancer were
diagnosed; ER and PR status was obtained in 2,062 of these cases. No association was observed between CLA intake and the incidence of breast cancer, overall or ER/PR-defined. ER/PR status indicates how well a breast cancer patient may respond to hormone therapy. Relative risks of all invasive tumors and ER/PR-defined tumors were consistent among all CLA intake deciles.

While the results of the study show no association between CLA intake and breast cancer risk, this might be due to relatively low CLA intake by the study population. As noted by the authors, studies reporting a reduction in tumor incidence relied upon much higher doses of CLA.

**Considerations for Future Research**

Some of the conflicting research surrounding CLA’s impact on tumorigenesis may be attributed to the low dosage tested and the short testing duration of previous studies. Additionally, studies involving human subjects are limited, primarily focusing on *in vitro*, *in vivo*, and animal research.

The uncertainty regarding CLA’s antitumorigenic effects may be resolved by testing higher CLA doses for a longer period of time in human subjects. A proposed future research objective is to determine whether CLA’s effects on inflammatory markers hsCRP and TNF-α, along with enzymes MMP-2, MMP-9, and S14, LPL and FASN, improve when administered to breast cancer and rectal cancer patients at a dose of 15g/d, for a duration of 60 days.

The randomized, double-blind, placebo-controlled pilot study will involve breast cancer and rectal cancer patients. The independent variable in this study is the CLA supplement; the dependent variables are the inflammatory markers and enzymes. Patients will be blindly assigned to either the CLA group or the placebo group. The CLA group will take capsules providing a total of 15g CLA daily in a 50:50 ratio of c9, t11 and t10, c12 isomers. Fasting blood samples will be obtained prior to and upon completion of the intervention. From the blood samples, 7 factors will be assessed: TNF-α, hsCRP, MMP-9, MMP-2, S14, LPL, and FASN.
Results from this proposed study may indicate whether CLA displays greater antitumorigenic effects when administered at increased dosage and duration than previously tested on human subjects.

**Lay Summary**

Linoleic acid is an 18-carbon essential fatty acid, possessing two double bonds at the 9 and 12 carbon atoms that are separated by two single bonds. Conjugated linoleic acid (CLA) isomers result when the two double bonds are no longer separated by two single bonds. The most abundant, and thus most researched, forms are the cis-9, trans-11 and trans-10, cis-12 configurations. Recent studies suggest that CLA may be an effective supplemental treatment option for patients with certain types of cancer, including rectal cancer and invasive non-metastatic breast cancer. CLA appears to significantly affect expression of a variety of inflammatory markers and enzymes involved in cancer cell proliferation. More extensive research is needed, however, to confirm CLA’s antitumorigenic properties in human subjects.
Works Cited


